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The use of morphometrics in entomological surveillance of sylvatic foci of *Triatoma infestans* in Bolivia

J.P. Dujardin [a,*], H. Bermudez [b], C.J. Schofield [c]

[a] *UMR CNRS-ORSTOM 9926, ORSTOM, BP 5045, 911 Av. Agropolis, Montpellier cedex-1, France*
[b] *CUMETROP, Facultad de Medicine, Universidad Mayor de San Simon, Cochabamba, Bolivia*
[c] *Department of Medical Parasitology, London School of Hygiene and Tropical Medicine, London WC1 E7HT, UK*

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Abstract

Jamach'uma (Cochabamba, Bolivia) is a small village surrounded by sylvatic foci of *Triatoma infestans*. Houses in the village were also infested with *T. infestans*, and were sprayed in December 1992 as part of a Chagas disease vector control trial. Ten months later the houses were found to be again infested with a few fifth instar nymphs of *T. infestans*. These nymphs were compared by seven head measurements with 36 fifth instar nymphs collected from houses in Jamach'uma before treatment, and with two sets of nymphs originating from the surrounding sylvatic foci: eight specimens collected in 1992 and nine specimens collected in 1995. The results are discussed in relation to the possible mechanisms of the apparent reinfestation: recrudescence of a residual domestic population or reinvasion of the houses from surrounding sylvatic foci. Quantitative comparisons support the former hypothesis. © 1997 Elsevier Science B.V.

Keywords: Triatoma infestans; Sylvatic focus; Morphometrics; Chagas disease; Bolivia

* Corresponding author. Persent address: IBBA, c/o Embajada de Francia, CP717, La Paz, Bolivia

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1. Introduction

Control of Chagas disease vectors relies primarily on spraying infested dwellings with pyrethroid insecticides. After the initial intervention however, it is important to continue entomological surveillance so that any new infestations can be selectively retreated (Schofield, 1994). In Bolivia, as in neighbouring countries of the Southern Cone, the main domestic vector of Chagas disease is *Triatoma infestans* (Klug). This species is almost exclusively domestic except for small sylvatic foci in the Cochabamba region of central Bolivia. The sylvatic foci are invariably rockpiles where wild rodents seem to be the main sylvatic hosts (Dujardin et al., 1987).

Jamach'uma is a small village close to the city of Cochabamba. Houses in the village were heavily infested with *T. infestans*, and the village is surrounded by sylvatic foci of *T. infestans*, with the nearest about 1 km from the houses (Dujardin et al., 1987; Bermudez et al., 1993). As part of a Chagas disease vector control trial, houses in Jamach'uma were sprayed with a pyrethroid insecticide (deltamethrin) in December 1992, which appeared to eliminate the domestic infestations. Ten months later, however (in October 1993), during the regular entomological surveillance after insecticide spraying, a total of ten fifth stage nymphs of *T. infestans* were found in the houses. The reappearance of domestic vectors could be due to immigrant bugs, in which case the nearest and most probable source would be the sylvatic foci. On the other hand, reinfestation could have arisen from individuals that had survived the initial insecticide treatment. These two hypotheses were explored by morphometric comparisons.

2. Materials and methods

2.1. The insects

Specimens of *T. infestans* were collected in the village of Jamach'uma, close to the city of Cochabamba (Bolivia), and in the surrounding sylvatic foci. The domestic specimens were collected at Jamach'uma village in 1992, before insecticide spraying (D92), and 10 months later in 1993, with the latter thus representing the reinfesting population (R93). The sylvatic foci were burrows of rodents (*Galea musteloides*) in rockpile, where *T. infestans* was collected in 1992 (S92) and 1995 (S95). The total sample comprised 63 fifth instar nymphs: eight specimens in sample S92 (one nest of *G. musteloides*), 36 in sample D92, nine in sample S95 (another nest of *G. musteloides*) and ten in sample R93. The head and abdomen of these specimens were preserved in ethanol at $-20°C$.

2.2. Head morphometrics

Fifth instar nymphs were considered separately according to their origin and year of capture. Seven measurements were taken for the head of each specimen (Fig. 1): IE-inner distance between eyes (synthlipis), AO-anteocular distance, PO-postocular

distance (excluding neck), WE-dorsal width of eye, AT-length of antenniferous tubercle, R2-length of second rostral segment and R3-length of third rostral segment. All measurements were made at 25 × or 40 × magnification using a monocular micrometre, by the same investigator.

2.3. Numerical analysis

Means and standard errors were calculated after appropriate transformations of the measurement units (Appendix A). A Mantel test was applied as a nonparamet-

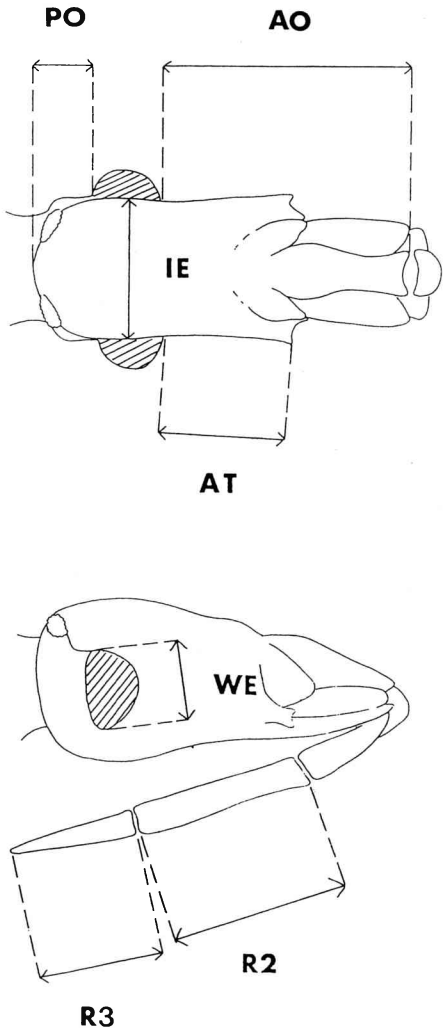

Fig. 1. Lateral (bottom) and dorsal (top) aspects of the head of fifth instar nymph of *Triatoma infestans*, to show measurements taken.

Table 1
Correlation between two distance matrices (Mantel test on Z value)

Design matrix composition	7 variables	1 variable (PO)	Number of random matching
S92, D92, R93, S95	0.000	0.002	500
S92 and R93	0.002	0.010	500
D92 and R93	0.744	0.928	500
S95 and R93	0.000	0.000	500

A dissimilarity matrix and a design matrix, as a non parametric ANOVA. Elements of the dissimilarity matrix are absolute differences between variates. The design matrix matches the structure of the dissimilarity matrix, having zeros in the within-group submatrices and ones elsewhere (Sokal and Rohlf, 1995). Values of the first two columns are the proportion of simulation $Z >= \text{Obs}$. The third column specifies the number of permutations which were run.

ric ANOVA on the whole sample (S92, D92, R93 and S95), as well as on each pair comparing the reinfesting specimens (R93) with each of the other groups (S92, D92 or S95) (Table 1). This test estimates the correlation between the dissimilarity matrix (absolute difference between individuals) and a design matrix indicating the within and between comparisons (Sokal and Rohlf, 1995). For the dissimilarity matrix, we used the absolute inter-individual differences either from the whole set of measurements, or from the post-ocular region (PO) alone (Table 2).

To equalize variances among groups and variables, data were log-transformed prior to the following statistical analysis (Yoccoz, 1993). Two conventional canonical variate analyses (CVA) were performed on the log-transformed data. First, it was performed on known populations (S92, D92, S95), and reinfesting nymphs were then introduced as supplementary data. Next, it was performed on the whole sample, including the reinfesting nymphs. From this latter CVA, the Mahalanobis distances allowed computation of a UPGMA tree (Fig. 2, top). Since different periods and sites of capture probably induced size differences, we verified this

Table 2
The first principal component as a general size variable

Variable	PC-1	r
IE	0.20664	0.5249
AO	0.33177	0.8380
PO	0.64752	0.8667
WE	0.33670	0.5961
AT	0.44807	0.7779
R2	0.26133	0.5635
R3	0.21336	0.4900

Criterion after Strauss (1985) allowing one to interpret the first principal component (PC1) as a general size variable after principal component analysis (PCA) involving seven head characters for 63 fifth stage nymphs of *Triatoma infestans* from domestic (D92), sylvatic (S92, S95) and unknown origin (R93). PC-1 = coefficients of the first principal component (all positive), r = correlation coefficients between the first eigenvector (PC-1) and the head characters. All r were positive and significant at $P<0.0001$.

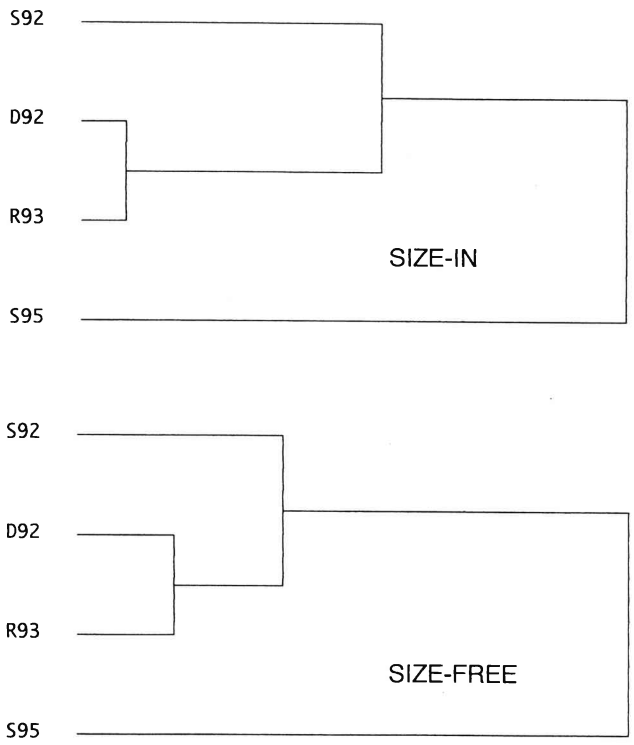

Fig. 2. UPGMA dendrograms derived from Mahalanobis distances after size-in (top) and size-free (bottom) canonical variate analysis (CVA), between four samples of *Triatoma infestans*. S92 = 8 fifth instar sylvatic nymphs collected in one nest of *Galea musteloides* in 1992 and D92 = 36 fifth instar domestic nymphs coming from Jamach'uma. Both S92 and D92 were collected before treatment of the village. R93 = 10 fifth instar nymphs of *T. infestans* newly infesting Jamach'uma, found after 10 months of entomological surveillance; S95 = 9 fifth instar sylvatic nymphs collected in 1995 in another nest of the rodent *G. musteloides*.

analysis also after removing the size effects (Dos Reis et al., 1990; Yoccoz, 1993). After examining the first factor (PC-1) of the covariance-matrix based principal component analysis to demonstrate its acceptability as a multivariate variable of size (Strauss, 1985; Table 2), it was used to compute expected values, and the residuals were submitted to a size-free CVA (Hutcheson et al., 1995). The Mahalanobis distances matrix computed from the size-free CVA was also converted into a UPGMA tree (Fig. 2, bottom). The significance of each CVA was estimated by a between-groups permutation test (Chessel and Dolédec, 1992), and by the Wilks statistics (Wilks, 1932; see also Tomassone et al., 1988) on the first discriminant function (Table 3). The statistics were performed using the STATA (Computing Resource Center, 1992. Stata Reference Manual: Release 3. 5th ed. Santa Monica, CA; Hamilton, 1993), ADE 4.1 (Chessel and Dolédec, 1992), MacDendro (Thioulouse, 1989) and STATITCF packages (Tomassone, 1988).

3. Results

Using either the whole set of measurements, or only the PO, the Mantel test showed that reinfesting specimens (R93) differed from sylvatic ones ($P < 0.010$), from either 1992 (S92) or 1995 (S95), but did not differ significantly from the original domestic specimens (D92) (Table 1).

The canonical variate analyses (CVA) were all significant ($P < 0.002$, Table 3). When introduced as supplementary data in a conventional CVA, nine reinfesting nymphs (out of ten) were classified with D92, and one with S92 (detailed results not shown). When R93 was introduced as a 'distinct' group in the CVA (Table 3, second and third lines), the low percentage of correct classification was due to the many classification errors between R93 and D92, the probabilities however remained highly significant due to the differences between sylvatic (S92, S95) and domestic specimens (D92, R93). In the UPGMA representation derived from the Mahalanobis distances (Fig. 2), reinfesting specimens (R93) were grouped with the original domestic ones (D92). This was true whether the data were scaled for size (Fig. 2, bottom) or not (Fig. 2, top).

4. Discussion

Ten months after spraying Jamach'uma, fifth stage nymphs were found in the houses, but not adults. At first sight, the hypothesis of nymphs being sylvatic migrants is not credible because nymphs lack wings. However, passive transportation of juvenile stages, by people or animals, is not uncommon in *T. infestans*. As a consequence, this criterion alone—the developmental stage of the reinfesting population—is not a definitive argument for rejecting the hypothesis of immigrant bugs. The metric characters of these nymphs are therefore presented here as an additional character to help clarify the source of the apparent reinfestations.

Table 3
Size-in and size free canonical variate analyses of heads comparisons

Samples	*P*w	*P*p	Classified
S92, S95, D92 (SI)	0.000	0.000	81.1
S92, S95, D92, R93 (SI)	0.000	0.000	57.1
S92, S95, D92, R93 (SF)	0.000	0.002	57.1

Pw refers to significance of the Wilks statistics (Wilks, 1932), which is provided by the STATITCF software (Tomassone, 1988). *P*p is the occurence of an eigenvalue as high or higher than the observed one after 500 between-group permutations (ADE 4.0 software, Chessel and Dolédec, 1992). The analysis without reinfesting specimens included in the data (samples D92, S92 and S95, first line) was used to introduce reinfesting nymphs (R93) as supplementary data. Classified indicates the proportion of individuals which have been correctly attributed to their respective group by the model (STATITCF software). The last two analysis were used to compute Mahalanobis distances from which dendrograms were constructed (Fig. 2).

Using Mantel tests, either with all the measurements or only with the PO distance, it was not possible to distinguish reinfesting nymphs (R93) from the original domestic population (D92) (Table 1). The PO region deserves particular mention here because we previously found that it was generally larger in adult stages (male and female) of sylvatic *T. infestans*, and could be proposed as a tentative diagnostic metric between ecotopes in this area (Dujardin et al., unpublished data). Mahalanobis distances derived from conventional, size-in CVA confirmed the similarity of reinfesting nymphs with those living at Jamach'uma before insecticide spraying (D92), and suggest that they are derived from the same domestic populations (Fig. 2, top).

However, size differences—or similarities—could arise from microenvironmental factors, differing from 1992 to 95 and generating different cohorts within the same population (Yoccoz, 1993). The statistical procedure used here for removing size differences, which is also recommended to avoid spurious results due to sampling artefacts (Dos Reis et al., 1990), allows the partitioning of environmental (size related differences) from evolutionary influences (Hutcheson et al., 1995). The comparison between size-in (Fig. 2, top) and size-free CVA (Fig. 2, bottom) showed that the relationships between groups were not size dependent, giving more confidence in assigning the reinfesting nymphs (R93) to the original domestic population (D92).

The differences between reinfesting and sylvatic nymphs, as well as the similarity between reinfesting and domestic nymphs, lend support to the hypothesis of a residual domestic population surviving after the insecticide application. Since the latter consisted of fifth stage nymphs captured 10 month after the spraying, they probably survived the insecticide spraying as eggs or first instar nymphs. This seems plausible because these life stages often lie deeply hidden in the crevices of walls, presumably protected against the effect of the insecticide.

Although we cannot definitively rule out the idea that some of the reinfesting nymphs may have had a sylvatic origin (note the results on reinfesting nymphs as supplementary data), three arguments are consistent with the hypothesis of no regular migrants, or exceptional migrants, between sylvatic and domestic ecotopes in this area: (1) the delay (10 months) between insecticide spraying and the reinfestation; (2) the stage (fifth instar nymphs) of the reinfesting population; and (3) their metric characteristics.

Our results are thus in agreement with the existence of a residual domestic population at Jamach'uma as the most likely explanation for the apparent reinfestation. This hypothesis was suggested in another area of Bolivia by isoenzyme analysis (Dujardin et al., 1996) and in Uruguay by head and thorax morphometry (Casini et al., 1995).

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Appendix A

Morphometric values (microns) of seven head characters (Fig. 1) in 63 fifth instar nymphs of *Triatoma infestans* from four captures (see Section 2). Number of heads measured (*N*), mean (Mean) and S.D., minimum (Min) and maximum (Max). The coefficient of variation (LogCV) was computed on log-transformed data.

Var.	Groups	*N*	Mean	S.D.	Min	Max	LogCV
IE	S92	8	1500	60	1440	1600	0.5477
	D92	36	1462	54	1360	1560	0.5121
	R93	10	1452	42	1360	1520	0.4047
	S95	9	1409	39	1360	1480	0.3787
AO	S92	8	2695	123	2600	2960	0.5594
	D92	36	2623	93	2400	2840	0.4551
	R93	10	2596	85	2480	2720	0.4158
	S95	9	2656	118	2400	2760	0.5790
PO	S92	8	797	85	720	940	1.5350
	D92	36	725	42	620	800	0.8788
	R93	10	723	31	700	800	0.6377
	S95	9	797	29	740	840	0.550
WE	S92	8	895	52	840	1000	0.8381
	D92	36	871	49	720	960	0.8492
	R93	10	864	43	800	920	0.7421
	S95	9	871	44	800	920	0.7453
AT	S92	8	1375	64	1320	1480	0.6376
	D92	36	1332	71	1120	1480	0.7591
	R93	10	1302	58	1200	1400	0.6290
	S95	9	1362	99	1240	1560	0.9936
R2	S92	8	2350	126	2200	2600	0.6780
	D92	36	2263	82	2040	2400	0.4763
	R93	10	2216	79	2120	2400	0.4550
	S95	9	2178	110	2000	2320	0.6622
R3	S92	8	1171	63	1080	1280	0.7607
	D92	36	1168	46	1080	1280	0.5566
	R93	10	1189	47	1080	1240	0.5747
	S95	9	1196	55	1120	1280	0.6465

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